

schematic microfluidic networks **748**. FIG. **3C-3** shows a plan view from the top of the cartridge, showing that each sample inlet **750** and sample chamber communicates with a separate microfluidic network. Other aspects of multi-sample microfluidic cartridges, such as communication with thermal actuator networks, may be accomplished for the example of FIG. **3C** as further described herein, as would be understood by one of ordinary skill in the art. The embodiment of FIG. **3C** may also utilize a mechanical key such as shown and described with respect to embodiments in FIGS. **3A** and **3B**.

#### Microfluidic Cartridge

[**0069**] Microfluidic cartridges as described herein, may adopt a number of different configurations of components without deviating from the spirit of the methods of analysis as described elsewhere herein. Such cartridges are configured to accept, separately, a sample and reagents, to lyse the sample, introduce the sample into a microfluidic network, and deliver an extract containing polynucleotides to an outlet. For example, an exemplary embodiment is found depicted in FIG. **1** of U.S. provisional application Ser. No. 60/726,066, filed Oct. 11, 2005 and incorporated herein by reference.

[**0070**] Referring to FIG. **4**, an exemplary multi-sample microfluidic cartridge **200** is shown in cross-section. The following description pertains to a single cartridge or lane as found in the multi-sample cartridge. Cartridge **200** includes first and second layers **205**, and **209**. First layer **205** functions as a microfluidic substrate and a microfluidic network is found inside. Within first layer there may be a further layer **207**, permitting various components of a microfluidic network **201** to be elevated with respect to one another. Second layer **209** is often referred to as a microfluidic substrate because it has one or more holes in it that align with and communicate with vents in the microfluidic substrate. On the exterior surface of the first layer **205** is typically a protective laminate coating **206**.

[**0071**] Microfluidic component **201** is configured to accept and to prepare a sample containing one or more polynucleotides. Cartridge **200** typically prepares a sample by lysing cells within the sample, and releasing the one or more polynucleotides in a form suitable for subsequent analysis. Cartridge **200** may also increase the concentration of one or more polynucleotides and/or reduce the concentration of inhibitors relative to the concentration of the one or more polynucleotides in the sample.

[**0072**] Microfluidic cartridge **200** can be fabricated as desired, preferably by injection moulding. Typically, layers **205**, **207**, and **209** are formed of a polymeric material. Elements of component **201** are typically formed by molding (e.g., by injection molding) layers **207**, **205**. Layer **206** is typically a flexible polymeric material (e.g., a laminate) that is secured (e.g., adhesively and/or thermally) to layer **205** to seal elements of component **201**. Layers **205** and **209** may be secured to one another using adhesive.

[**0073**] Exemplary cartridge **200** also comprises a bulk lysis chamber **264** and a waste chamber a **269**. Preferably these two chambers are fabricated as a single piece, and separated by a barrier **199**. FIG. **4B** shows an exemplary exploded view of cartridge **200** with various of its components as typically fabricated. Interior funnels **197** are

optional and have ramped surfaces that cause liquid to flow downwards under force of gravity towards exit hole **282**. Side walls **195** of the funnels are optional and facilitate certain fabrication processes.

[**0074**] Cartridge **200** further comprises a sample inlet **202** by which sample material, preferably in the form of a liquid solution containing cells, can be introduced into bulk lysis chamber **264**. Two luer are shown, offset with respect to one another, and situated on adjacent cartridges or lanes of multi-sample cartridge **200**, in FIG. **4**. Preferably, sample inlet **202** takes the form of a luer having a one-way valve **203**. The sample inlet directs sample into bulk lysis chamber **264**, in which cells in the sample are lysed, when in contact with bulk lysis reagent pellets (not shown) in chamber **264**, or by application of heat to chamber **264**, or by a combination of both application of heat and contact with reagent pellets. Sample inlet **202** preferably includes a one-way valve that permits material (e.g., sample material and gas) to enter chamber **264** but limits (e.g., prevents) material from exiting chamber **264** by the sample inlet. Typically, the inlet includes a fitting (e.g., a luer fitting) configured to mate with a sample input device (e.g., a syringe) to form a gas-tight seal. Lysis chamber **264** typically has a volume of about 5 milliliters or less (e.g., about 4 milliliters or less). Prior to use, lysis chamber **264** is typically filled with a gas (e.g., compressed air **263**).

[**0075**] In general, the volume of sample introduced is smaller than the total volume of lysing chamber **264**. For example, the volume of sample may be about 50% or less (e.g., about 35% or less, about 30% or less) of the total volume of chamber **264**. A typical sample has a volume of about 3 milliliters or less (e.g., about 2.0 milliliters or less, or about 1.5 milliliters or less). A volume of gas (e.g., air) is generally introduced to chamber **264** along with the sample. Typically, the volume of gas introduced is about 50% or less (e.g., about 35% or less, about 30% or less) of the total volume of chamber **264**. The volume of sample and gas combine to pressurize the gas already present within chamber **264**.

[**0076**] Bulk lysis reagent pellets when used preferably contain one or more particles such as DNA capture beads (not shown) that are designed to retain polynucleotide molecules. Particles are preferably modified with at least one ligand that retains polynucleotides (e.g., preferentially as compared to inhibitors). Exemplary ligands for preferentially retaining polynucleotides include, for example, polyamides (e.g., poly-cationic polyamides such as poly-L-lysine, poly-D-lysine, poly-DL-ornithine, and poly-ethylene-imine, polyhistidine). Ligands such as polyboronic acid can also be used for retaining RNA. Other ligands include, for example, intercalators, poly-intercalators, minor groove binders, polyamines (e.g., spermidine), homopolymers and copolymers comprising a plurality of amino acids, and combinations thereof. In some embodiments, the ligands have an average molecular weight of at least about 5,000 Da (e.g., at least about 7,500 Da, or at least about 15,000 Da). In some embodiments, the ligands have an average molecular weight of about 50,000 Da or less (e.g., about 35,000, or less, about 27,500 Da or less). In some embodiments, the ligand is a poly-lysine ligand attached to the particle surface by an amide bond.

[**0077**] In certain embodiments, the ligands are resistant to enzymatic degradation, such as degradation by protease